Role of the low-affinity glucocorticoid receptor in the regulation of behavior and energy metabolism in the migratory red knot Calidris canutus islandica

Landys, M M; Piersma, T; Ramenofsky, M; Wingfield, John C.

Published in:
Physiological and Biochemical Zoology

DOI:
10.1086/420942

Role of the Low-Affinity Glucocorticoid Receptor in the Regulation of Behavior and Energy Metabolism in the Migratory Red Knot *Calidris canutus islandica*

Mėta M. Landys[1,*]
Theunis Piersma[2,3]
Marilyn Ramenofsky[1]
John C. Wingfield[1]
[1]Department of Biology, University of Washington, Seattle, Washington 98195; [2]Department of Marine Ecology and Evolution, Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands; [3]Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Accepted 11/24/03

ABSTRACT

Plasma corticosterone increases in association with migratory flight in the red knot *Calidris canutus islandica*, suggesting that corticosterone may promote migratory activity and/or energy mobilization in this species. This hypothesis is supported by general effects of glucocorticoids, which include stimulation of locomotion and the mobilization of energy depots. We experimentally examined the role of elevated corticosterone levels in the migratory red knot by comparing foraging behavior, flight frequency, and plasma metabolites between vehicle-injected controls and birds treated with RU486, an antagonist to the genomic low-affinity glucocorticoid receptor (GR). We predicted that RU486 treatment would interfere with energy mobilization. However, we expected no effects on flight activity because recent studies suggest that glucocorticoids affect locomotion through a nongenomic receptor. Finally, because glucocorticoids exert permissive effects on food intake, we postulated that RU486 treatment in the red knot would interfere with feeding. Results were consistent with the latter prediction, suggesting that the GR participates in the promotion of hyperphagia, the intense feeding state that is characteristic of the migratory condition. RU486 treatment did not affect flight frequency, suggesting that corticosterone may support migratory activity through a receptor other than the GR. Energy metabolism (as determined through plasma metabolites) was also unaffected by RU486, possibly because energetic demands experienced by captive birds were low.

* Corresponding author. Present address: Department of Biology, University of Oslo, P.O. Box 1050, Blindern, N-0316 Oslo, Norway; e-mail: meta.landys@bio.uio.no.

Physiological and Biochemical Zoology 77(4):658–668. 2004. 1522-2152/2004/7704-3097$15.00

Introduction

Elevated levels of corticosterone have been noted during migration in a variety of bird species (Holberton et al. 1996; Romero et al. 1997; Holberton 1999; Piersma et al. 2000). Corticosterone appears to increase specifically in association with migratory flight (Landys-Ciannelli et al. 2002; Landys et al. 2004*b*) and therefore may promote processes associated with this substage of migration, such as migratory activity and energy mobilization. Such a prediction is consistent with past research (Dolnik and Blyumental 1967; Meier and Martin 1971), in which corticosterone was shown to contribute to the regulation of migratory restlessness, the intense and persistent movements that characterize birds in a migratory condition. However, conclusions from these latter studies should be interpreted with some caution because results were obtained from birds that received exogenous glucocorticoids. This technique often elevates glucocorticoid levels to pharmacological concentrations (Gray et al. 1990; Astheimer et al. 1992) that may misrepresent stage-specific levels. During migration, elevations in plasma corticosterone are only slight and do not approach maximal concentrations (e.g., Schwabl et al. 1991; Gwinner et al. 1992; Romero et al. 1997; Tsipoura et al. 1999; Mizrahi et al. 2001; Landys-Ciannelli et al. 2002; Landys et al. 2004*b*).

Landys et al. (2004*a*) recently examined the role of corticosterone in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*. Effects of corticosterone on behavior and energy metabolism during spring migration were determined by comparing vehicle-injected controls to birds treated with RU486, an antagonist to the genomic low-affinity glucocorticoid receptor (GR). Thus, this study addressed the role of an endogenous increase in corticosterone appropriate to the life-history stage of migration. Landys et al. (2004*a*) found that fasting birds treated with RU486 were unable to mobilize lipids to the same degree as untreated controls. Such results suggest that corticosterone may act through the GR to

promote fat breakdown during periods of high-energy demand, such as during migratory flight. In addition, because treatment with RU486 resulted in decreased food intake, Landys et al. (2004*a*) suggested that the GR may play a permissive role in the expression of hyperphagia, the intense feeding state that characterizes migration.

As shown in other species (Landys-Ciannelli et al. 2002; Landys et al. 2004*b*), migrating red knots *Calidris canutus islandica* display elevated levels of corticosterone specifically in association with the substage of migratory flight. For example, when held in captivity on their wintering grounds, red knots show an increase in plasma corticosterone only after having attained a body mass similar to that of departing conspecifics (Piersma et al. 2000). Also, free-living red knots display elevated corticosterone at the conclusion of a long bout of migratory flight, as they arrive at their breeding grounds in the North American Arctic (Reneerkens et al. 2002). The correlation between corticosterone and migratory flight in this species suggests that corticosterone may be involved in the onset and regulation of migratory movements and correlated physiological processes. To experimentally determine the role of elevated corticosterone in the migratory red knot, we treated captive birds with RU486 or with vehicle during spring migration and then compared activity and food intake between treatment groups. Birds were examined during the migratory peak in body mass, when plasma corticosterone is typically elevated in association with departure. We also investigated effects of RU486 treatment on plasma metabolite concentrations.

On the basis of results from previous studies (Landys et al. 2004*a*), we predicted that RU486 treatment would decrease food intake in migratory knots. Glucocorticoids have been postulated to exert permissive effects on feeding (King 1987; Dallman et al. 1993) and therefore may be required for the expression of hyperphagia. Because glucocorticoids also stimulate glucose production (Davison et al. 1983; Simon 1984; Dallman et al. 1989; Santana et al. 1995) and the breakdown of lipid stores (Mukherjee and Mukherjee 1973; Nazir et al. 1988; Sellers et al. 1988; Dallman et al. 1993; Hadley 1999), we also predicted that RU486 treatment in migratory knots would inhibit energy mobilization, as expressed by decreased plasma levels of glucose and free fatty acids. However, we predicted that RU486 treatment would not affect activity levels. Evidence to date suggests that glucocorticoids affect locomotion and possibly migratory restlessness through a receptor other than the GR (Moore and Orchinik 1994; Sandi et al. 1996; Breuner et al. 1998; Landys et al. 2004*a*).

Methods

Animals

The red knot subspecies investigated in this study travels approximately 3,000 km from wintering areas in coastal Europe to breeding grounds on the high Arctic tundra in northeast Canada and northern Greenland (Davidson and Wilson 1992; Piersma et al. 2004). Birds undertake a single refueling stop in Iceland and therefore complete migration in only two bouts of flight. Because birds overfly open ocean during both bouts, they must be able to precisely regulate energy deposition and the breakdown of energy stores during fueling and flight if travel is to be completed successfully.

Wintering birds were captured with mistnets in the Wadden Sea of the Netherlands, Germany, and Denmark in September 1999 and 2000. Birds were housed at the Royal Netherlands Institute for Sea Research on the island of Texel. Knots were held in outdoor flight aviaries (seven or eight birds per aviary) complete with an artificial saltwater mudflat in which they expressed behavior typical of shorebirds, such as probing. Birds were exposed to a natural photoperiod at all times and were fed ad lib. with trout pellets. Birds were removed from aviaries once every week so that aviaries could be cleaned and were weighed and checked for general health status at this time.

Corroboration Study

Piersma et al. (2000) and Reneerkens et al. (2002) suggested that corticosterone levels in migrating red knots increase specifically in association with migratory flight. To corroborate these findings, we first investigated plasma levels of corticosterone in captive birds during spring migration (May–June) in the year 2000. Blood samples for the determination of plasma corticosterone were collected on May 2, May 16, June 6, and June 20 from seven individual knots that were captured in the fall of 1999. Knots in this study were kept in a single aviary.

To collect blood samples, we punctured the alar wing vein with a sterile 23-gauge needle and drew the pooling blood into heparinized microhematocrit capillary tubes. Baseline corticosterone levels that reflect undisturbed corticosterone concentrations were evaluated from blood samples collected within 3 min of entry into the aviary (Wingfield et al. 1982). Blood was collected at similar times during each sampling day to avoid diel corticosterone differences among samples. To determine how baseline corticosterone levels compare with stress-induced concentrations, birds were again sampled 30 min after disturbance according to a standardized handling-stress protocol (Wingfield et al. 1995). Birds were held in individual containers between blood sampling procedures. Body mass of birds was determined in association with each bleeding period.

We used one-way repeated measures ANOVA tests to investigate changes in body mass and in baseline and stress-induced levels of corticosterone over the 2-mo sampling period. Specific differences among sampling days were evaluated with Tukey post hoc tests. We used a Pearson product moment correlation on all samples to determine the strength and direction of the relationship between body mass and baseline corticosterone. In birds that had reached peak body mass, we compared baseline corticosterone concentrations and stress-induced levels with a

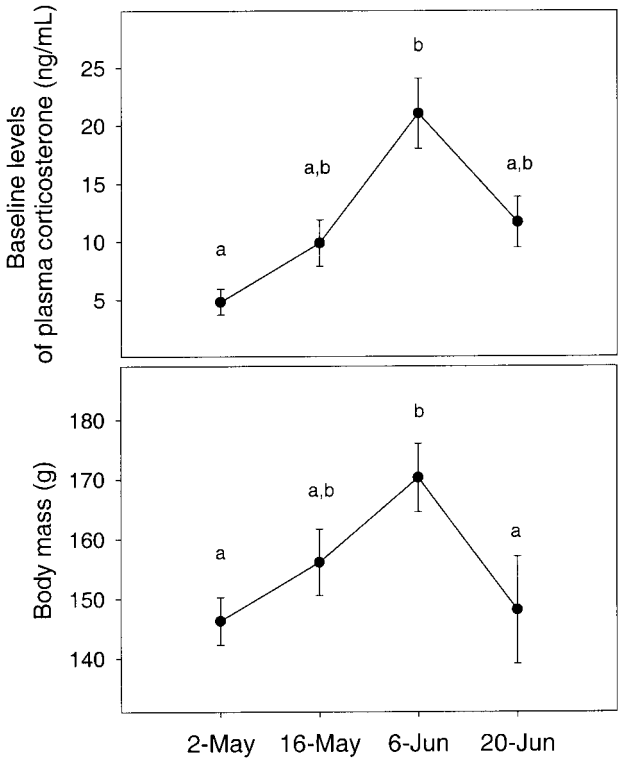

Figure 1. Body mass (g) and baseline plasma corticosterone (ng/mL) of captive red knots during the period of spring migration (May–June). Corticosterone levels were evaluated from blood samples collected in under 3 min of entry into aviaries and reflect undisturbed concentrations. Elevations in plasma corticosterone coincide with the peak in body mass. Different letters indicate significant differences among sampling periods. Sample size includes seven birds. Error bars represent SEs of the mean.

paired *t*-test. *P* values were Bonferroni adjusted. All corticosterone data were $\log_{10}$ transformed before use in statistical analyses.

Inhibition of the Low-Affinity Glucocorticoid Receptor

We experimentally investigated the role of an elevated corticosterone baseline in captive red knots that were captured in autumn of 2000. Birds were examined during the migratory peak in body mass, when plasma corticosterone was expected to be elevated. Birds were divided into three treatment groups consisting of 10 animals each: (1) vehicle-injected controls, (2) birds administered with 10 mg/kg of RU486 (mifepristone; Sigma), and (3) birds administered with 50 mg/kg of RU486. Dose was determined from previous studies conducted in mammals (Hinz and Hirschelman 2000; McKeown et al. 2000). RU486 was suspended in vehicle (peanut oil; Hain Celestial) via sonication and was administered by subcutaneous injection. Injections were performed in the morning. The 30 birds involved in this study were randomly divided among four aviaries, with seven to eight birds per aviary. In any one week, experiments were conducted on birds from only two aviaries.

Increasing evidence suggests that as in mammals, RU486 administration in birds antagonizes the low-affinity GR (Breuner and Orchinik 2001; Koch et al. 2002; Breuner et al. 2003; Landys et al. 2004*a*). RU486 also inhibits the progesterone receptor. Thus, results should be interpreted with some caution. However, we maintain that effects of RU486 in this study should be representative of action at the level of the GR because we measured behavioral and physiological responses that are classically attributed to glucocorticoids rather than to progesterone (Hadley 1999; Nelson 2000).

Data Collection

We tested for effects of RU486 by comparing behavior of individual birds 1 d before and 1 d after injections. Birds were marked with unique color bands for identification. The following behaviors were monitored: resting, standing, flying, walking, feeding from a food tray, drinking, bathing, preening, and probing. Although no food was available on either the mudflat or the aviary floor, probing behavior (a repeated insertion of the bill into the mudflat or floor crevices) persisted. Probing behavior is almost an obligatory exploration routine for this species and does not require reinforcement by food retrieval (although ingestion of food pellets usually leads to probing). In fact, red knots possess a specialized bill tip to indirectly locate hard objects in soft sediments (Piersma et al. 1998).

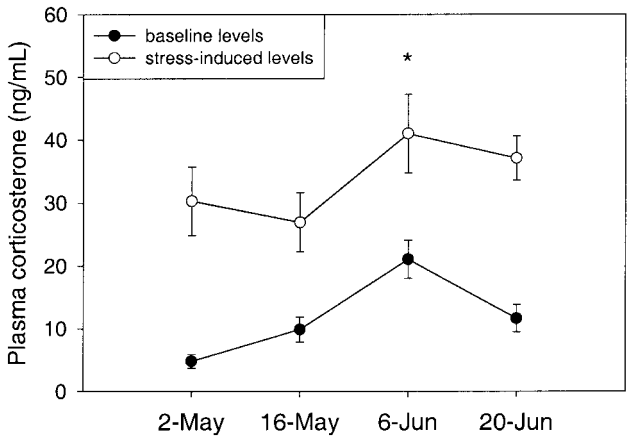

Figure 2. Stress-induced plasma corticosterone (ng/mL) of captive red knots during the period of spring migration (May–June). Stress-induced levels were evaluated from blood samples collected after 30 min of handling. Levels were not different among sampling periods. For comparison, we show baseline concentrations (taken from Fig. 1). At the time of the body mass peak, stress-induced levels were significantly higher than baseline concentrations, as indicated by the asterisk. Sample size includes seven birds. Error bars represent SEs of the mean.

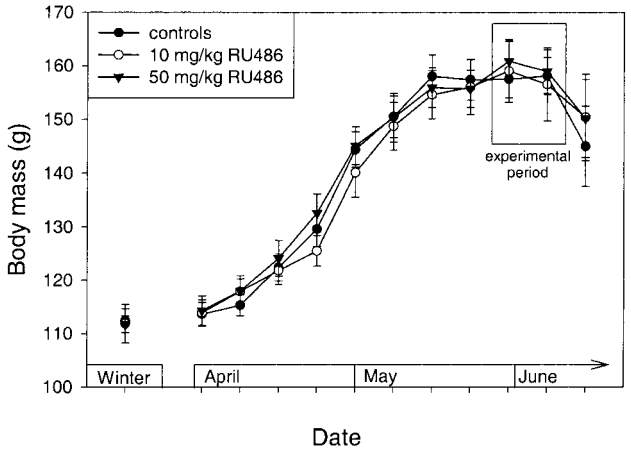

Figure 3. Body mass (g) of experimental red knots during the period of spring migration. Birds were weighed every 7 d (as indicated by ticks on the *X*-axis) and were divided according to future treatment group. Body mass in all treatment groups began to increase in April and reached peak levels toward the end of May. Experiments were conducted when controls, 10 mg/kg RU486 birds, and 50 mg/kg RU486 birds were within 97.1% ± 0.9%, 98.7% ± 0.5%, and 98.0% ± 0.5% of their peak in body mass, respectively. Ten birds were included in each treatment group. Error bars represent SEs of the mean.

Behavioral data were collected by scanning individual birds once every minute. We recorded the number of times a bird expressed a certain behavior during at least 100 min on each sampling day. The fraction of time birds spent engaged in each behavior was calculated by dividing the number of times a behavior was expressed by the total number of observations. Data for the most common behaviors (resting, standing, walking, preening, and probing) were arcsin square root transformed and entered into a principal components model. The first factor generated by the model was used as a compressed score for behavior. Effects of RU486 on behavioral scores were tested with a two-way repeated measures ANOVA. Sampling day (before or after treatment) was included as a repeated factor.

We also recorded the total number of take-off flights initiated from the cage floor on each sampling day to determine the effects of RU486 on flight frequency (flights/h). To examine how treatment affected food intake, feeding behavior was recorded on video and analyzed for number of foraging trips undertaken to the food tray and number of trout pellets eaten per hour. Although knots had reached maximum body mass, we maintain that birds were still in a hyperphagic state typical of the migratory condition: intense feeding is required to retain peak body mass during spring migration. First, fueled captive knots quickly lose body mass if disturbed, and second, all captive birds lose mass at a rapid rate 1–2 wk after mass peak. Differences in flight frequency, foraging trip frequency, and rate of food pellet intake among the three treatment groups were statistically examined with two-way repeated measures ANOVAs. Sampling day (before or after treatment) was included as a repeated factor. Data was $\log_{10}$ transformed for use in statistical analyses. If significant differences were found, paired *t*-tests with Bonferroni-adjusted *P* values were used to determine the groups within which treatment had an effect. To verify that experimental birds were in migratory condition, we compared the flight frequency and foraging trip frequency of individual birds between winter (January 2001) and the period of spring migration (at the time of the body mass peak) with paired *t*-tests.

Blood Sampling Procedures

Blood samples for the determination of plasma corticosterone were first collected from experimental birds during winter (January 2001). Blood samples were again collected in early June during the mass peak both immediately before injections and 2 d after injections, that is, at the conclusion of the experiment. Baseline corticosterone levels were evaluated from blood samples that were collected within 3 min of entry into aviaries.

To verify that corticosterone levels in experimental birds increased in association with the period of migration, we compared baseline plasma corticosterone between wintering birds and birds at the time of the body mass peak in spring (before injections) with a two-way repeated measures ANOVA. Treatment group (controls, 10 mg/kg RU486 birds, or 50 mg/kg RU486 birds) was included as a factor.

At the conclusion of the experiment, effects of RU486 on plasma corticosterone were examined by comparing baseline

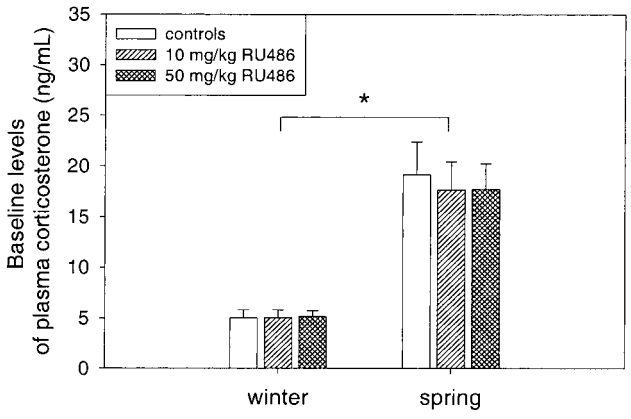

Figure 4. Baseline plasma corticosterone (ng/mL) of experimental red knots during winter and also at the time of their body mass peak in spring. Corticosterone levels were evaluated from blood samples collected in under 3 min of entry into aviaries and reflect undisturbed corticosterone concentrations. Birds are divided according to future treatment group. All treatment groups showed higher plasma levels of corticosterone during spring migration than during winter, as indicated by the asterisk. Ten birds were included in each group. Error bars represent SEs of the mean.

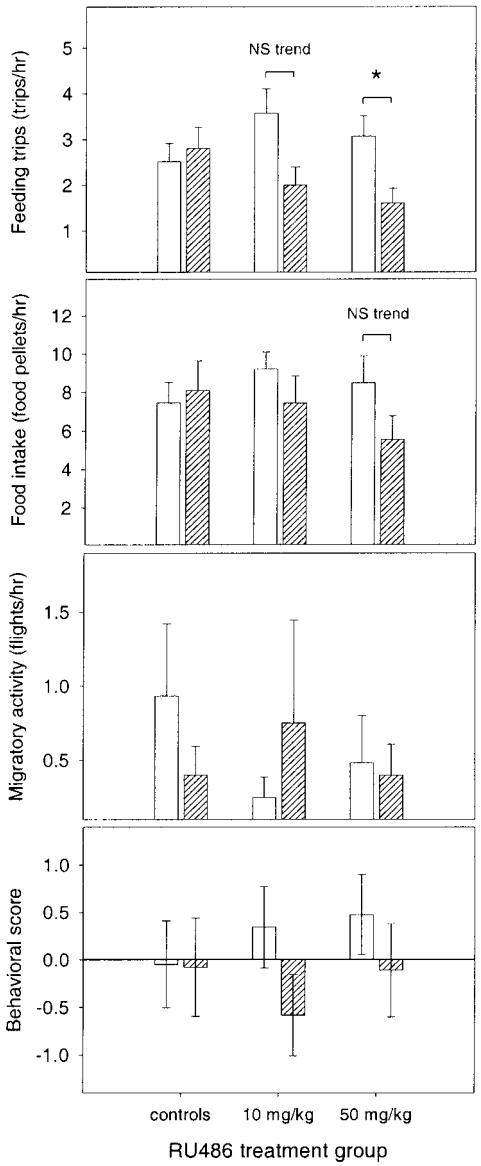

Figure 5. Feeding trip frequency (trips/h), rate of food intake (pellets/h), flight activity (flights/h), and behavioral score of experimental red knots, that is, controls, 10 mg/kg RU486 birds, and 50 mg/kg RU486 birds. Open bars represent data collected before treatment; hatched bars represent data collected after treatment. Asterisk indicates significant differences between treatment days. Ten birds were included in each treatment group. Error bars represent SEs of the mean.

corticosterone levels (posttreatment) among treatment groups with a one-way ANOVA. Posttreatment plasma samples were also evaluated for five plasma metabolites: triglycerides, free fatty acids, glycerol, uric acid, and glucose. Triglycerides are the storage form of lipids. They are synthesized in the liver and are transported to peripheral tissues for deposition into fat bodies but can also enter the blood via dietary absorption (Robinson 1970). Free fatty acids and glycerol are released when triglycerides are hydrolyzed and indicate lipid mobilization from adipose tissue (Scow and Chernick 1970; Hurley et al. 1986; Elia et al. 1987). Uric acid results from the breakdown of protein that originates from bodily tissue or the diet (Mori and George 1978; Robin et al. 1987; Lindgård et al. 1992). Concentrations of each of the five plasma metabolites were compared among the three treatment groups with a one-way ANOVA. All metabolite data were $\log_{10}$ transformed before statistical analysis.

Blood Assays

Blood samples were centrifuged immediately after collection. Plasma for the determination of corticosterone levels and metabolites was stored in microcentrifuge tubes at −20°C and −80°C, respectively. Corticosterone concentrations were determined with direct radioimmunoassay (RIA) as described in Wingfield et al. (1992). Briefly, plasma samples of 20 μL were combined with 180 μL distilled H_2O. Then, 2000 cpm ^{3}H-corticosterone (Perkin Elmer; catalog NET-399) were added and allowed to equilibrate overnight at 4°C for the determination of percent recovery of steroid. Steroids were extracted for 2 h with 4 mL redistilled dichloromethane and dried under nitrogen gas at 37°C. Dried extracts were resuspended in 550 μL phosphate-buffered saline (0.1% gelatin). Duplicate samples of 200 μL were used for the RIA. Corticosterone concentration was calculated from a standard curve that ranged from 7.8 to 2,000 pg. Of the remaining resuspended extract, 100 μL was used to determine the percent steroid recovered after extraction. Corticosterone concentrations were adjusted to account for percent steroid lost during extraction. We set the limits of detectability at 15 pg. As calculated from known standards, interassay variation was 14.6%. As previously determined from 10 known standards that were analyzed by M. M. Landys in a separate assay, intra-assay variation was 8.7%.

Metabolite concentrations in blood plasma were determined on a powerwave 340 × microplate spectrophotometer (BioTec Instruments; Guglielmo et al. 2002). Assays were run in 400-μL flat-bottom, 96-well polystyrene microtiter plates (NUNC). Glycerol and triglycerides were measured sequentially by endpoint assay (Sigma, GPO-Trinder reagents A and B; 5 μL plasma, 240 μL reagent A, 60 μL reagent B). Glucose was measured by endpoint assay (Sigma, INFINITY glucose reagent; 3 μL sample, 300 μL reagent). Free fatty acids were measured by endpoint assay (WAKO Diagnostics; 3 μL sample, 120 μL reagent A, 240 μL reagent B). Uric acid was measured by endpoint assay (WAKO Diagnostics; 5 μL sample, 300 μL reagent).

Table 1: Component loadings of the behavioral variables generated by the first factor of the principal components model

Component Loadings	PC1
Resting	.837
Standing	.422
Walking	−.832
Preening	.023
Probing	−.690

Note. Only the most frequent behaviors were entered into the model: resting, standing, walking, preening, and probing. We used the first principal component factor (PC1) as a compressed score for behavior.

Results

Corroboration Study

Body mass of captive red knots changed with sampling day during spring migration, that is, during May and June ($F_{3,18} = 5.948$, $P = 0.005$; Fig. 1). Birds exhibited significantly greater body mass on June 6 than on May 2 or June 20 ($P < 0.05$).

Sampling day also significantly affected baseline levels of corticosterone ($F_{3,18} = 8.062$, $P = 0.002$). Plasma corticosterone was significantly higher on June 6 than on May 2 ($P < 0.05$). The June 6 elevation in baseline corticosterone coincided with the peak in body mass (Fig. 1); that is, plasma corticosterone was highest in the most fully fueled birds ($R^2 = 0.395$, $n = 28$, $P = 0.038$). Stress-induced levels of corticosterone did not change with sampling period ($F_{3,18} = 2.785$, $P = 0.071$).

Although plasma corticosterone showed a positive correlation with body mass during spring migration, it was not elevated to maximal concentrations. Handling induced a significant increase in plasma corticosterone at the time of the body mass peak ($t = -3.151$, df = 6, $P = 0.040$). In fact, stress-induced levels were at least two times greater than baseline concentrations (Fig. 2).

Investigation of RU486 Effects in Migratory Red Knots

Body mass of experimental birds in all three treatment groups increased between January and June, in concert with the onset of spring migration (Fig. 3). Birds expressed significantly higher flight frequency during spring migration than during winter ($t = 2.627$, df = 29, $P = 0.014$; 0.56 ± 0.20 and 0.05 ± 0.03 flights/h, respectively). However, in spite of the mass peak during spring, foraging trip frequency was similar between seasons ($t = 0.825$, df = 29, $P = 0.416$; 3.8 ± 0.3 and 3.5 ± 0.5 trips/h, respectively). Experiments in spring were conducted when controls, 10mg/kg RU486 birds, and 50 mg/kg RU486 birds were within $97.1\% \pm 0.9\%$, $98.7\% \pm 0.5\%$, and $98.0\% \pm 0.5\%$ of their peak in body mass, respectively.

Baseline plasma corticosterone in experimental birds was higher at the time of the spring experiments than during winter ($F_{1,27} = 96.359$, $P < 0.001$; Fig. 4). Baseline corticosterone was not different among RU486 groups before treatment ($F_{2,27} = 0.0387$, $P = 0.962$), suggesting that all three groups displayed similar preexperimental elevations. The interaction between treatment group and season was not significant ($F_{2,27} = 0.137$, $P = 0.873$).

Frequency of visits to the food tray was greater before treatment than after treatment ($F_{1,27} = 16.385$, $P < 0.001$). Although the average frequency of foraging trips was similar among RU486 treatment groups ($F_{2,27} = 0.303$, $P = 0.741$), a significant interaction term suggests that in response to treatment, the pattern of change among groups was different ($F_{2,27} = 6.309$, $P = 0.006$; Fig. 5). Specifically, treatment decreased foraging trip frequency in birds that received 50 mg/kg of RU486 ($t = 3.596$, df = 9, $P = 0.024$). Birds treated with 10 mg/kg of RU486 showed a strong trend for decreased foraging frequency ($t = 3.067$, df = 9, $P = 0.052$), and foraging trip frequency was unaffected by treatment in controls ($t = -0.913$, df = 9, $P > 1.0$).

Birds consumed food pellets at a similar rate before and after treatment ($F_{1,27} = 2.764$, $P = 0.108$). Rate of food intake was not different among RU486 treatment groups ($F_{2,27} = 0.376$,

Table 2: Behavioral budget of red knots according to treatment group (controls, 10 mg/kg RU486 birds, and 50 mg/kg RU486 birds) during spring migration

	Pretreatment			Posttreatment		
Behavior	Controls	10 mg/kg	50 mg/kg	Controls	10 mg/kg	50 mg/kg
Resting	31.9 ± 5.3	40.4 ± 5.5	39.7 ± 4.7	27.2 ± 3.1	28.1 ± 2.6	31.8 ± 3.8
Standing	19.7 ± 2.9	16.4 ± 2.6	19.2 ± 2.3	24.6 ± 3.8	17.3 ± 2.4	22.4 ± 2.7
Walking	15.3 ± 3.5	16.8 ± 2.9	18.2 ± 3.2	21.0 ± 5.0	26.3 ± 5.2	23.3 ± 5.0
Preening	15.7 ± 4.1	12.3 ± 1.1	12.8 ± 1.9	12.8 ± 2.3	12.7 ± 2.9	9.0 ± 1.5
Probing	11.8 ± 2.1	9.3 ± 2.4	7.0 ± 2.1	9.1 ± 1.9	9.3 ± 1.8	8.7 ± 1.6

Note. Behavior was evaluated before and after treatment. The behavior of individual birds was recorded once every minute during >100 min of each sampling day. The percentage of time birds spent engaged in each behavior was calculated from these values. Ten birds were included in each treatment group. Data are shown as average percentages ± SE.

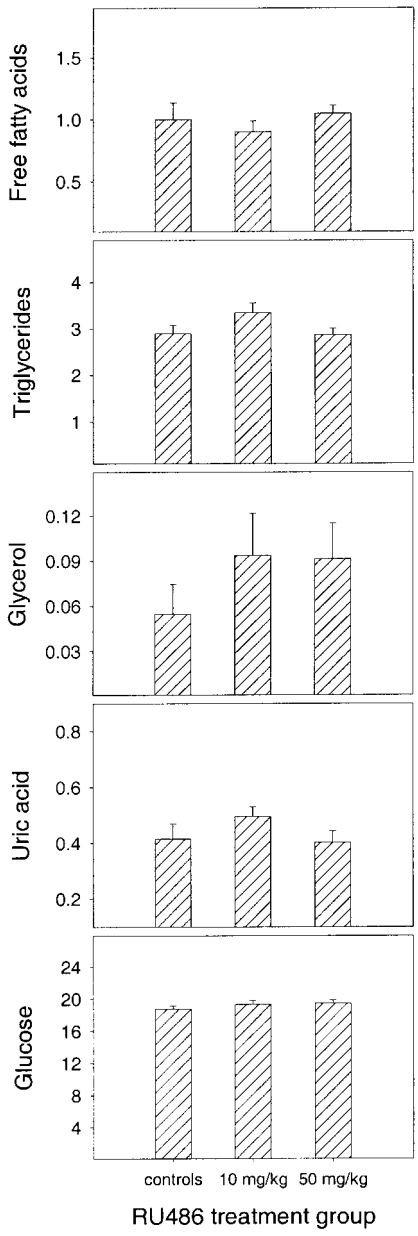

Figure 6. Plasma metabolites (mmol/L) of experimental red knots during spring migration. Birds are divided according to treatment: controls, 10 mg/kg RU486 birds, and 50 mg/kg RU486 birds. Concentrations of five metabolites were evaluated: free fatty acids, triglycerides, glycerol, uric acid, and glucose. RU486 treatment did not significantly affect plasma levels of any of these five metabolites. Ten birds were included in each treatment group. Error bars represent SEs of the mean.

$P = 0.690$), and the interaction between treatment and sampling day was not significant ($F_{2,27} = 1.689$, $P = 0.204$). However, if controls are compared only against the group receiving the highest dose of RU486 (50 mg/kg), there is a weak trend for treatment to change food intake differently between these two groups (interaction effect: $F_{1,18} = 3.043$, $P = 0.098$; Fig. 5).

Birds displayed the same frequency of take-off flights before and after treatment ($F_{1,27} = 0.0160$, $P = 0.900$). Flight frequency was not different among RU486 treatment groups ($F_{2,27} = 0.163$, $P = 0.850$), and the interaction was not significant ($F_{2,27} = 0.958$, $P = 0.396$; Fig. 5).

The first principal component factor used to compress the five most commonly expressed behaviors (resting, standing, walking, preening, and probing) into one behavioral score explained 41.0% of the total variance. Component loadings for the first principal components factor are shown in Table 1. Behavioral scores were similar before and after treatment ($F_{1,27} = 2.610$, $P = 0.118$) and were not different among the three treatment groups ($F_{2,27} = 0.192$, $P = 0.827$; Fig. 5; Table 2). The interaction between treatment group and treatment day was not significant ($F_{2,27} = 0.673$, $P = 0.518$).

After treatment, groups displayed similar plasma levels of all measured metabolites: free fatty acids ($F_{2,27} = 0.656$, $P = 0.527$), triglycerides ($F_{2,27} = 1.930$, $P = 0.165$), glycerol ($F_{2,27} = 0.856$, $P = 0.436$), glucose ($F_{2,27} = 0.522$, $P = 0.599$), and uric acid ($F_{2,27} = 1.263$, $P = 0.299$; Fig. 6). Baseline levels of plasma corticosterone were also unaffected by RU486 treatment ($F_{2,27} = 0.0465$, $P = 0.955$; Fig. 7).

Discussion

The captive red knots investigated in this study clearly increased in body mass during the period of spring migration and displayed increased flight activity, suggesting that they had entered into the migratory condition. However, foraging trip frequency in spring was similar to that displayed during winter, suggesting similar food intake rates between seasons. Wiersma and Piersma (1994) have indicated that maintenance metabolism of knots wintering in the Dutch Wadden Sea is close to maximal. Thus, high feeding rates during winter may be critical to support increased thermoregulation costs, which are replaced by the costs of migratory fueling later in the year.

Consistent with previous work described in Piersma et al. (2000), baseline levels of plasma corticosterone in spring birds were elevated in association with the peak in body mass at the time when wild conspecifics typically initiate migratory flight. However, as in other migrating species (e.g., Schwabl et al. 1991; Gwinner et al. 1992; Romero et al. 1997; Tsipoura et al. 1999; Mizrahi et al. 2001; Landys-Ciannelli et al. 2002), plasma corticosterone in red knots was not elevated to maximal stress concentrations. Thus, if corticosterone participates in the regulation of migration, it probably does so at an intermediate level (reviewed in Landys 2003).

To determine how the low-affinity GR regulates migratory processes in the presence of endogenous corticosterone elevations, we investigated effects of RU486 treatment on activity, food intake, and energy metabolism of captive red knots at the time of their migratory peak in body mass. We found that RU486 treatment decreased the expression of foraging behavior:

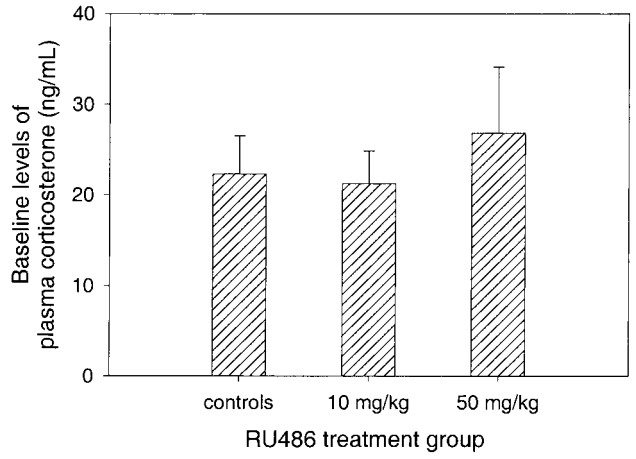

Figure 7. Baseline plasma corticosterone (ng/mL) of migratory red knots after treatment. Corticosterone levels were evaluated from blood samples collected in under 3 min of entry into aviaries and reflect undisturbed corticosterone concentrations. RU486 treatment did not affect plasma corticosterone. Ten birds were included in each treatment group. Error bars represent SEs of the mean.

knots conducted fewer visits to the food tray when given an increasingly greater dose of RU486. We are confident that effects of RU486 on food intake were not pharmacological: general behavior of birds was not affected by treatment. Thus, as already proposed for a passerine species (Landys et al. 2004*a*), binding of the GR may be necessary for the expression of hyperphagia.

Although inhibition of corticosterone binding decreased foraging behavior in the red knot, we suggest that feeding is not regulated through changes in the level of plasma corticosterone. Periods of migratory fueling are characterized by relatively low corticosterone levels (Landys-Ciannelli et al. 2002; Landys et al. 2004*b*). Even in the red knots examined here, plasma corticosterone was comparatively lower during mass gain than during mass peak. We propose that mass gain may instead be supported by an increase in GR number. Evidence from house sparrows (*Passer domesticus*) suggests that the GR population does vary seasonally (Breuner and Orchinik 2001). A variable GR population is also suggested by data from the white-crowned sparrow, in which RU486 treatment affects food intake during spring (when GR number is predicted to be high) but not during winter (Landys et al. 2004*a*).

Irrespective of how hyperphagia is supported—through increased plasma corticosterone or increased GR number—it is important to note that activation of the GR probably influences hyperphagia only in a permissive capacity and does not directly stimulate a feeding response (King 1987; Dallman et al. 1993). GR binding may regulate food intake by altering responsivity to other hormones or neurotransmitters, such as neuropeptide Y or norepinephrine. For example, both norepinephrine (Leibowitz et al. 1984; Tempel and Leibowitz 1993) and neuropeptide Y (Stanley et al. 1989; Tempel and Leibowitz 1993; Zakrzewska et al. 1999) induce food intake in intact rats. This feeding response is abolished by adrenalectomy and can be restored by administration of glucocorticoids.

Although GR binding may be required for the expression of feeding behavior during migration, the timing of elevated corticosterone levels to the fully fueled state (Fig. 1) suggests that corticosterone's primary role during migration centers around processes associated with migratory flight. For example, migration-associated elevations in corticosterone may assist in the mobilization of energy stores to fuel long-distance movements (Landys et al. 2004*a*) and/or may promote intense and persistent activity during travel (Dolnik and Blyumental 1967; Meier and Martin 1971). Corticosterone may even influence departure decisions and choice of travel direction (Lõhmus et al. 2003).

To determine whether binding of the GR by plasma corticosterone affects activity in migratory red knots, we compared the frequency of take-off flights between RU486-treated birds and vehicle-injected controls. Although glucocorticoids are known to affect general locomotion (Veldhuis et al. 1982; Astheimer et al. 1992), RU486 treatment did not affect flight frequency in examined knots. This result is consistent with previous work in another migrant (Landys et al. 2004*a*), suggesting that if plasma corticosterone affects the expression of activity during migration, then it does so through a receptor other than the GR. Because an immediate behavioral effect after glucocorticoid administration has been noted in a variety of animals (Moore and Orchinik 1994; Sandi et al. 1996), including a migratory bird (Breuner et al. 1998), it is possible that elevated levels of corticosterone affect migratory activity through a nongenomic receptor.

We also investigated effects of RU486 treatment on concentrations of five plasma metabolites. Contrary to our predictions, RU486 did not affect energy metabolism in migratory red knots: plasma levels of all measured metabolites were similar between treatment groups. Because birds were allowed to feed before the collection of metabolite data, they were in a state of positive energy balance. Past research suggests that endogenous elevations in corticosterone affect energy mobilization only during periods of energy demand. For example, in the white-crowned sparrow, RU486 affects lipid mobilization only during a fast (Landys et al. 2004*a*). Also, handling stress in the European starling *Sturnus vulgaris* increases glucose levels only at night, when birds have not been actively feeding (Remage-Healy and Romero 2000, 2001). Thus, in migrants, metabolic effects of an elevated baseline may become prominent only as birds complete fueling, so that as they begin to experience the energetic demands associated with departure and extended flight, lipid stores can be effectively mobilized.

We found that RU486 treatment in the red knot did not affect corticosterone levels (Fig. 7). RU486 treatment in mammals often causes elevations in plasma corticosterone due to inhibition of negative feedback in the hypothalamus and pi-

tuitary (Langley and York 1990; Cooney and Dinan 1996). Thus, data from this study suggest that negative feedback in the red knot may be independent of GR occupation. On the other hand, because RU486 action at transcriptional sites may depend on the presence of specific coactivators or corepressors (McKenna and O'Malley 2000), RU486 may not act as a GR antagonist in the red knot hypothalamus or pituitary. Mechanisms underlying the regulation of negative feedback may be species specific or condition specific, especially because some studies have reported decreased plasma corticosterone after RU486 treatment, for example, in mammals (e.g., Laue et al. 1988) and in a passerine migrant (Landys et al. 2004*a*). Regardless, the lack of an effect on plasma corticosterone in knots simplifies the interpretation of results because observed effects on food intake are likely due to RU486 action in the brain rather than to differences in circulating levels of corticosterone.

In summary, results from this study demonstrate that RU486 treatment suppresses foraging behavior of red knots during spring migration. Similar effects have been found in a passerine migrant, the white-crowned sparrow (Landys et al. 2004*a*), suggesting that hyperphagia may be controlled similarly among migratory species. Second, we found no effects of RU486 treatment on plasma metabolite levels, possibly because birds were not subjected to energetic stress before investigation. Finally, RU486 did not affect the frequency of take-off flights, suggesting that the GR does not mediate migratory activity. Thus, hyperphagia and migratory restlessness, the two behaviors that characterize migration, may be regulated by different mechanisms, as previously suggested by King and Farner (1963). Future studies will hopefully elucidate the mechanisms underlying the control of migratory restlessness and will begin to address the question as to how corticosterone may interact with other hormones or neurotransmitters to regulate behavioral and physiological processes during migration.

Acknowledgments

We gratefully acknowledge members of the Piersma laboratory for assistance with blood collection; Lynn Erckmann for helping with the radioimmunoassay; Chris Guglielmo for the invitation to do metabolite assays at the University of Montana, Missoula; and Bernard Spaans and Anne Dekinga for tracking seasonal changes in body mass of captive red knots and for bird care. Studies were supported by a Personal Impulse for Research Groups with New Ideas for Excellent Research grant awarded to T.P. from the Netherlands Organization for Scientific Research and by a National Science Foundation grant from the Office of Polar Programs awarded to J.C.W.

Literature Cited

Astheimer L.B., W.A. Buttemer, and J.C. Wingfield. 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scand 23:355–365.

Breuner C.W., A.L. Greenberg, and J.C. Wingfield. 1998. Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Gen Comp Endocrinol 111:386–394.

Breuner C.W. and M. Orchinik. 2001. Seasonal regulation of membrane and intracellular corticosteroid receptors in the house sparrow brain. J Neuroendocrinol 13:412–420.

Breuner C.W., M. Orchinik, T.P. Hahn, S.L. Meddle, I.T. Moore, N.T. Owen-Ashley, T.S. Sperry, and J.C. Wingfield. 2003. Differential mechanisms for plasticity of the stress response across latitudinal gradients. Am J Physiol 285:R594–R600.

Cooney J.M. and T.G. Dinan. 1996. Type II (glucocorticoid) receptors mediate fast-feedback inhibition of the hypothalamic-pituitary-adrenal axis in man. Life Sci 59:1981–1988.

Dallman M.F., D.N. Darlington, S. Suemaru, C.S. Cascio, and N. Levin. 1989. Corticosteroids in homeostasis. Acta Physiol Scand 583(suppl.):27–34.

Dallman M.F., A.M. Strack, S.F. Akana, M.J. Bradbury, E.S. Hanson, K.A. Scribner, and M. Smith. 1993. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Frontiers Neuroendocrinol 14:303–347.

Davidson N.C. and J.R. Wilson. 1992. The migration system of European-wintering knots *Calidris canutus islandica*. Wader Study Group Bull 64(suppl.):39–51.

Davison T.F., J. Rea, and J.G. Rowell. 1983. Effects of dietary corticosterone on the growth and metabolism of immature *Gallus domesticus*. Gen Comp Endocrinol 50:463–468.

Dolnik V.R. and T.I. Blyumental. 1967. Autumnal premigratory and migratory periods in the chaffinch (*Fringilla coelebs coelebs*) and some other temperate zone passerine birds. Condor 69:435–468.

Elia M., C. Zed, G. Neale, and G. Livesey. 1987. The energy cost of triglyceride–fatty acid recycling in non-obese subjects after an overnight fast and four days of starvation. Metabolism 36:251–255.

Gray J.M., D. Yarian, and M. Ramenofsky. 1990. Corticosterone, foraging behavior, and metabolism in dark-eyed juncos, *Junco hyemalis*. Gen Comp Endocrinol 79:375–384.

Guglielmo C.G., P.D. O'Hara, and T.D. Williams. 2002. Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living western sandpipers (*Calidris mauri*). Auk 119:437–445.

Gwinner E., M. Zeman, I. Schwabl-Benzinger, S. Jenni-Eiermann, L. Jenni, and H. Schwabl. 1992. Corticosterone levels of passerine birds during migratory flight. Naturwissenschaften 79:276–278.

Hadley M.E. 1999. Endocrinology. 5th ed. Prentice Hall, Upper Saddle River, N.J.

Hinz B. and R. Hirschelmann. 2000. Rapid non-genomic feedback effects of glucocorticoids on CRF-induced ACTH secretion in rats. Pharm Res 17:1273–1277.

Holberton R.L. 1999. Changes in patterns of corticosterone secretion concurrent with migratory fattening in a Neotropical migratory bird. Gen Comp Endocrinol 116:49–58.

Holberton R.L., J.D. Parrish, and J.C. Wingfield. 1996. Modulation of the adrenocortical stress response in Neotropical migrants during autumn migration. Auk 113:558–564.

Hurley B.F., P.M. Nemeth, W.H. Marcin III, J.M. Hagberg, G.P. Dalsky, and J.O. Holloszy. 1986. Muscle triglyceride utilization during exercise: effect of training. J Appl Physiol 60: 562–567.

King B.M. 1987. Glucocorticoids and hypothalamic obesity. Neurosci Biobehav Rev 12:29–37.

King J.R. and D.S. Farner. 1963. The relationship of fat deposition to Zugunruhe and migration. Condor 65:200–233.

Koch K.A., J.C. Wingfield, and J.D. Buntin. 2002. Glucocorticoids and parental hyperphagia in ring doves (*Streptopelia risoria*). Horm Behav 41:9–21.

Landys M.M. 2003. The Role of Baseline Corticosterone in the Regulation of Avian Migration. PhD diss. University of Washington.

Landys M.M., M. Ramenofsky, and J.C. Wingfield. 2004*a*. The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory white-crowned sparrow *Zonotrichia leucophrys gambelli.* J Exp Biol 207:143–154.

Landys M.M., J.C. Wingfield, and M. Ramenofsky. 2004*b*. Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys gambelli.* Horm Behav (in press).

Landys-Ciannelli M.M., M. Ramenosky, T. Piersma, J. Jukema, Castricum Ringing Group, and J.C. Wingfield. 2002. Baseline and stress-induced plasma corticosterone during long-distance migration in the bar-tailed godwit, *Limosa lapponica.* Physiol Biochem Zool 75:101–110.

Langley S.C. and D.A. York. 1990. Effects of antiglucocorticoid RU 486 on development of obesity in obese *fa/fa* Zucker rats. Am J Physiol 259:R539–R544.

Laue L., G.P. Chrousos, D.L. Loreiaux, K. Barnes, P. Munson, L. Nieman, and G. Schaison. 1988. The antiglucocorticoid and antiprogestin steroid RU486 suppresses the adrenocorticotropin response to ovine corticotropin releasing hormone in man. J Clin Endocrinol Metab 66:290–293.

Leibowitz S.F., C.R. Roland, L. Hor, and V. Squillari. 1984. Noradrenergic feeding elicited via the paraventricular nucleus is dependent on circulating corticosterone. Physiol Behav 32:857–864.

Lindgård K., K.A. Stokkan, Y. Le Maho, and R. Groscolas. 1992. Protein utilization during starvation in fat and lean Svalbard ptarmigan (*Lagopus mutus hyperboreus*). J Comp Physiol B 162:607–613.

Lõhmus M., R. Sandberg, R.L. Holberton, and F.R. Moore. 2003. Corticosterone levels in relation to migratory readiness in red-eyed vireos (*Vireo olivaceus*). Behav Ecol Sociobiol 54: 233–239.

McKenna N.J. and B.W. O'Malley. 2000. From ligand to response: generating diversity in nuclear receptor coregulator function. J Steroid Biochem Mol Biol 74:351–356.

McKeown K.J., J.R.G. Challis, C. Small, L. Adamson, A.D. Bocking, M. Fraser, D. Rurak, K.W. Riggs, and S.J. Lye. 2000. Altered fetal pituitary-adrenal function in the ovine fetus treated with RU486 and meloxicam, an inhibitor of prostaglandin synthase-II. Biol Reprod 63:1899–1904.

Meier A.H. and D.D. Martin. 1971. Temporal synergism of corticosterone and prolactin controlling fat storage in the white-throated sparrow, *Zonotrichia albicollis.* Gen Comp Endocrinol 17:311–318.

Mizrahi D.S., R.L. Holberton, and S.A. Gathreaux, Jr. 2001. Patterns of corticosterone secretion in migrating semipalmated sandpipers at a major spring stopover site. Auk 118: 79–91.

Moore F.L. and M. Orchinik. 1994. Membrane receptors for corticosterone: a mechanism for rapid behavioral responses in an amphibian. Horm Behav 28:512–519.

Mori J.G. and J.C. George. 1978. Seasonal changes in serum levels of certain metabolites, uric acid and calcium in the migratory Canada goose (*Branta canadensis interior*). Comp Biochem Physiol B 59:263–269.

Mukherjee S.P and C. Mukherjee. 1973. Control of rat adipose tissue metabolism by cortisone in relation to epinephrine. Am J Physiol 224:898–903.

Nazir M.I., H.A. Rizvl, and S.S. Ali. 1988. Effect of corticosterone on the lipid composition of adipose tissue, plasma and liver in a lizard. Pak J Sci Ind Res 31:706–710.

Nelson R.J. 2000. An Introduction to Behavioral Endocrinology. 2d ed. Sinauer, Sunderland, Mass.

Piersma T., J. Reneerkens, and M. Ramenofsky. 2000. Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? Gen Comp Endocrinol 120:118–126.

Piersma T., D.I. Rogers, P.M. González, L. Zwarts, L.J. Niles, I.-L. Serrano do Naschimento, C.D.T. Minton, and A.J. Baker. 2004. Fuel storage rates before northward flights in red knots worldwide: facing the severest ecological constraint in tropical intertidal environments? In R. Greenberg and P.P. Marra, eds. Birds of Two Worlds. Johns Hopkins University Press, Baltimore.

Piersma T., R. Van Aelst, K. Kurk, H. Berkhoudt, and L.R.M. Mass. 1998. A new pressure sensory mechanism for prey detection in birds: the use of principles of seabed dynamics? Proc R Soc Lond B Biol Sci 265:1377–1383.

Remage-Healy L. and L.M. Romero. 2000. Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): glucose. Gen Comp Endocrinol 119:60–68.

———. 2001. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. Am J Physiol 281:R994–R1003.

Reneerkens J., R.I.G. Morrison, M. Ramenofsky, T. Piersma, and J.C. Wingfield. 2002. Baseline and stress-induced levels of corticosterone during different life cycle substages in a shorebird on the high arctic breeding grounds. Physiol Biochem Zool 75:200–208.

Robin J.-P., Y. Cherel, H. Girard, A. Géloen, and Y. Le Maho. 1987. Uric acid and urea in relation to protein catabolism in long-term fasting geese. J Comp Physiol B 157:491–499.

Robinson D.S. 1970. The function of the plasma triglycerides in fatty acid transport. Pp. 51–105 in M. Florkin and E.H. Stotz, eds. Comprehensive Biochemistry. Vol. 18. Elsevier, Amsterdam.

Romero L.M., M. Ramenofsky, and J.C. Wingfield. 1997. Season and migration alters the corticosterone response to capture and handling in an arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelli*). Comp Biochem Physiol C 116:171–177.

Sandi C., C. Venero, and C. Gauze. 1996. Novelty-related rapid locomotor effects of corticosterone in rats. Eur J Neurosci 84:794–800.

Santana P., S.F. Akana, E.S. Hanson, A.M. Strack, R.J. Sebastian, and M.F. Dallman. 1995. Aldosterone and dexamethasone both stimulate energy acquisition whereas only the glucocorticoid alters energy storage. Endocrinology 136:2214–2222.

Schwabl H., F. Bairlein, and E. Gwinner. 1991. Basal and stress-induced corticosterone levels of garden warblers, *Sylvia borin*, during migration. J Comp Physiol B 161:576–580.

Scow R.O. and S.S. Chernick. 1970. Mobilization, transport, and utilization of free fatty acids. Pp. 19–50 in M. Florkin and E.H. Stotz, eds. Comprehensive Biochemistry. Vol. 18. Elsevier, Amsterdam.

Sellers T.L., A.W. Jaussi, H.T. Yang, R.W. Heninger, and W.W. Winder. 1988. Effects of exercise-induced increase in glucocorticoids on endurance in the rat. J Appl Physiol 65:173–178.

Simon J. 1984. Effects of daily corticosterone injections upon plasma glucose, insulin, uric acid and electrolytes and food intake pattern in the chicken. Diabetes Metab 10:211–217.

Stanley B.G., D. Lanthier, A.S. Chin, and S.F. Leibowitz. 1989. Suppression of neuropeptide Y–elicited eating by adrenalectomy or hypophysectomy: reversal with corticosterone. Brain Res 501:32–36.

Tempel D.L. and S.F. Leibowitz. 1993. Glucocorticoid receptors in PVN: interactions with NE, NPY and Gal in relation to feeding. Am J Physiol 265:E794–E800.

Tsipoura N., C.G. Scanes, and J. Burger. 1999. Corticosterone and growth hormone levels in shorebirds during spring and fall migration stopover. J Exp Zool 284:645–651.

Veldhuis H.D., E.R. De Kloet, I. Van Zoest, and B. Bohus. 1982. Adrenalectomy reduces exploratory activity in the rat: a specific role of corticosterone. Horm Behav 16:191–198.

Wiersma P. and T. Piersma. 1994. Effects of microhabitat, flocking, climate and migratory goal on energy expenditure in the annual cycle of red knots. Condor 96:257–279.

Wingfield J.C., K.M. O'Reilly, and L.B. Astheimer. 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. Am Zool 35:285–294.

Wingfield J.C., J.P. Smith, and D.S. Farner. 1982. Endocrine response of white-crowned sparrows to environmental stress. Condor 84:399–409.

Wingfield J.C., C.M. Vleck, and M.C. Moore. 1992. Seasonal changes in the adrenocortical response to stress in birds of the Sonoran Desert. J Exp Zool 264:419–428.

Zakrzewska K.E., A. Sainsbury, I. Cusin, J. Rouru, B. Jeanrenaud, and F. Rohner-Jeanrenaud. 1999. Selective dependence of intracerebroventricular neuropeptide Y–elicited effects on central glucocorticoids. Endocrinology 140:3183–3187.